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Results of a United States and Soviet Union Joint Project on Nervous System Effects of Microwave Radiation

by Clifford L. Mitchell,* Donald I. McRee,* N. John Peterson,* Hugh A. Tilson,* Mikhael G. Shandala,† Mikhael I. Rudnev,† Vyacheslav V. Varetskii,† and Mikhael I. Navakatikyan†

During the course of a formal program of cooperation between the United States and the Soviet Union concerning the biological effects of physical factors in the environment, it was concluded that duplicate projects should be initiated with the general goal of determining the most sensitive and valid test procedures for evaluating the effects of microwave radiation on the central nervous system. This report details an initial step in this direction. Male rats of the Fischer 344 strain were exposed or sham exposed to 10 mW/cm^2 continuous wave microwave radiation at 2.45 GHz for a period of 7 hr. Animals were subjected to behavioral, biochemical, or electrophysiological measurements during and/or immediately after exposure. Behavioral tests used were passive avoidance and activity in an open field. Biochemical measurements were ATPase (Na^+,K^+; Mg^{2+},Ca^{2+}) and K^+ alkaline phosphatase activities. Electrophysiological measurements consisted of EEG frequency analysis. Neither group observed a significant effect of microwave irradiation on open field activity. Both groups observed changes in variability of the data obtained using the passive avoidance procedure, but not in the same parameters. The U.S. group, but not the USSR group, found significantly less Na^+,K^+-ATPase activity in the microwave-exposed animals compared to the sham exposed animals. Both groups found incidences of statistically significant effects in the power spectral analysis of EEG frequency, but not at the same frequency. The failure of both groups to substantiate the results of the other reinforces our contention that such duplicate projects are important and necessary.

Introduction

A formal program of cooperation between the United States and the Soviet Union concerning the biological effects of physical factors in the environment has been in existence since 1975 (*1*). During a US-USSR workshop on nervous system effects of electromagnetic waves held in the US in 1982 (*2*), it was concluded that duplicate projects should be initiated with the general goal of determining the most sensitive and valid test procedures for evaluating the effects of microwave radiation on the central nervous system. Effects on the central nervous system and behavior have been the subject of greatest controversy in the entire field of bioeffects of microwave radiation (*3*). We believe this is due, at least in part, to a lack of unified methodological approaches and an inadequate data base for selecting the most appropriate methods. Moreover, there have been few attempts to verify many of the findings in other laboratories (*4*). It was for these reasons that this joint project was undertaken.

As an initial step in this direction, a protocol was developed for an experiment with short-term exposure using methods considered by the Soviet scientists to be sensitive in detecting microwave effects. Behavioral, biochemical, and electrophysiological investigations were included because concomitant measurements at different levels of neural organization aid in substantiating the validity of the results and are vital for understanding the mechanism of observed effects.

The behavioral tests chosen were simple examples of "naturalistic" (open field) and "learned" (passive avoidance) behaviors. On the basis of previous experience with

*Laboratory of Molecular and Integrative Neuroscience, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

†A. N. Marzeev Research Institute of General and Communal Health, Ministry of Health of Ukranian SSR, Kiev, USSR.

Address reprint requests to C. L. Mitchell, Laboratory of Molecular and Integrative Neuroscience, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

these tests, using Wistar rats (*5*), it was expected that differences between microwave-exposed and sham-exposed rats would be detected. For the biochemical studies we chose to study membrane-associated ATPases for several reasons. ATPases represent a basic element in the active transmembrane transfer of ions (*6*). Moreover, they bear interrelationships with each other since Na^+,K^+-ATPase activity is indirectly dependent on intracellular Ca^{2+} ion concentration. In turn, Ca^{2+} distribution is regulated, in part, by Mg^{2+},Ca^{2+}-ATPase (*6,7*). Also, Na^+,K^+-ATPase has been postulated to play a role in thermoregulation (*8*). The electrophysiological studies included spectral analysis of the electroencephalogram and averaged visual-evoked potentials. Preliminary studies indicated that both these parameters were affected by short-term exposure to microwave irradiation (V. V. Varetskii, unpublished results).

This report details the results obtained at the National Institute of Environmental Health Sciences (NIEHS) and the A. N. Marzeev Research Institute of General and Communal Hygiene (MRIGCH).

Methods

Subjects

Experiments were carried out using male rats of the Fischer 344 strain weighing 200 to 250 g. At NIEHS, the animals were obtained from Charles River Kingston Breeding Laboratory, Stone Ridge, NY. The animals were received at the Institute 1 week before placing them in the environmental chambers. The animal room was maintained at 21 ± 2°C and 50 to 60% humidity and on a day-night cycle from 0700 to 1900 (light) and 1900 to 0700 (dark). After 1 week the animals were moved to two environmental chambers (one chamber for exposure and the other for sham exposure), which were maintained at the conditions specified above. Food (NIH rat chow) and water were given *ad libitum* except during adaptation, exposure, and testing procedures.

Conditions at MRIGCH were similar except that the animals were obtained from their own colony. This colony was established in 1983 using animals obtained from Jackson Laboratories, London.

Analysis of the food showed that both groups of animals had a similar diet (Table 1).

Adaptation Procedure

Beginning 8 days prior to exposure, all animals experienced a daily 8 hr adaptation period in which they were housed in individual polypropylene plastic cages (28.6 cm long, 18 cm wide, and 13 cm high) inside the appropriate anechoic chamber (one for microwave exposure and one for sham exposure). This period was from 0830 to 1630. During this time the tops of the cages were removed and the animals were deprived of food and water. At all other times the animals remained in the individual cages but had food and water *ad libitum*. In order to keep the animal in the individual cages while the tops were removed, the cages were placed on a 30-cm high styrofoam support (Fig. 1).

Table 1. Analysis of rat chow.

Content	Percent by weight	
	US	USSR
Protein	18.5	19.6
Fat	4.5	2.6
Fiber	3.7	6.7
Ash	6.0	7.5
Phosphorus	0.93	0.71
Calcium	1.18	1.61

Exposure Procedure

At NIEHS, four animals were exposed and four animals were sham exposed following the 8-day adaptation period. The rats in the exposed group were exposed from above in the far field of a horn antenna to a power density of 10 mW/cm^2 of continuous wave 2.45 GHz microwave radiation for a period of 7 hr starting at 0900 and ending at 1600 (Fig. 1). The variation in incident power density from cage to cage did not vary more than ± 10%. These measurements were made at each cage location with all cages in place and inside each cage with all cages in place with a NBS Model B probe. During the exposure the chambers were maintained at a temperature of 21 ± 2°C and humidity of 50 ± 10%. The specific absorption rate (SAR) was measured using dead animals located at various orientations to the E field in an exposure cage. A calorimetric technique was used to make these measurements. The average whole-body SAR was found to be 2.7 mW/g when all orientations were considered (*9*).

Two animals from each of the exposed and sham-exposed groups were used for behavioral measurements, and the other two animals of each group were used for biochemical measurements. The experiment included 10

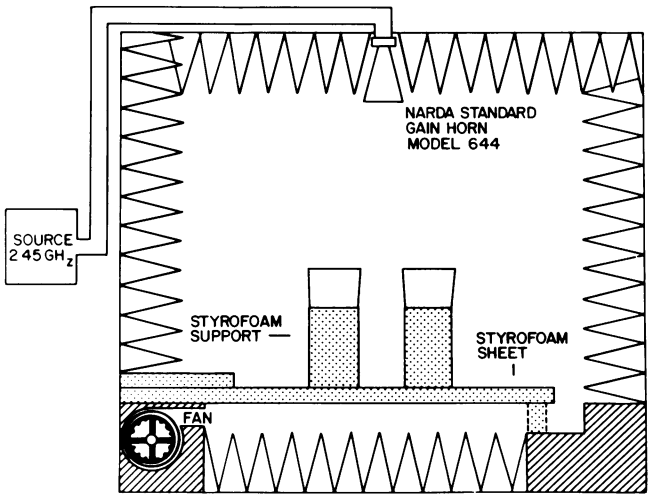

FIGURE 1. Experimental arrangements inside the environmental chamber.

replications, which resulted in 20 animals per group at the completion of the experiment.

At a later date the same procedure was followed except that only two animals were exposed and two were sham exposed at a time. Again, there were 20 animals in each group. These were used for electrophysiological measurements.

The procedure at MRIGCH was similar except that three animals were exposed and three were sham exposed at a time. The number of animals used for behavioral, biochemical, and electrophysiological measurments during any given exposure varied from 1 to 3. The experiment included 7 animals per group for behavioral analysis and 18 per group for both biochemical and electrophysiological analyses. Power density and SAR measurements yielded the same results as at NIEHS. Measurements were made by both the U.S. and the USSR investigators in the Soviet's exposure chambers and excellent agreement was obtained (*10,11*).

Behavioral Assessments

The behavioral tests used were passive avoidance and activity in an open field. The same rats were used in both tests. A diagram of the passive avoidance apparatus is shown in Figure 2.

Four days prior to microwave exposure all animals were tested for 3 consecutive days for a light avoidance response in the absence of footshock. The test duration was 3 min and performed between 1600 and 1630. The purpose of this procedure was to select only those animals for the experiment that entered the dark chamber and remained there for at least 30 sec one or more times during the three test periods. All animals met this criterion.

Passive avoidance training was performed between 1600 and 1630 on the day prior to exposure. Each animal was given a 3-min opportunity to avoid the light for periods up to 6 sec. If the rat spent more than 6 sec in the dark chamber it received a shock of 0.8 mA from aluminum rods composing the floor of the chambers. The parameters measured were: a) latency for light avoidance, b) time in the dark chamber, and c) entries into the dark chamber per session and latency to the first entry. In addition, the number of animals that avoided the shock on the retest day was recorded.

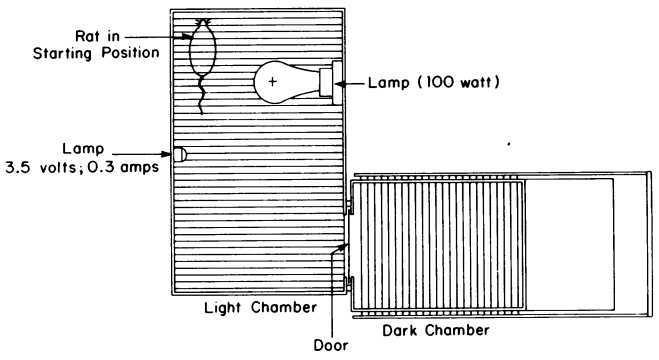

FIGURE 2. Diagram of the passive avoidance conditioned reflex apparatus, viewed from the top.

Immediately after exposure the rats used for behavior were placed in an open field of dimensions 1 m × 1 m with 30-cm high sides. The floor was divided into 20 cm × 20 cm squares alternating in color from white to blue. Indices were taken in three trials, each of 1 min duration. At the beginning of each trial the rat was placed under an opaque plastic bottomless box (cowl) in the center of the field (white square) for 15 to 20 sec. The box was made of colored Plexiglas. The dimensions of the box were 15 cm in length, 9 cm in width, 12 cm in height, with a 1 cm gap at the bottom of the long sides for the rat's tail. The nine center squares were designated the center of the field.

The following parameters were recorded: a) exploratory activity: the total number of squares crossed (crossed by the rat's back paws); b) central activity: the number of squares crossed in the center of the field after the rat has left the center once; and c) vertical activity: the number of times the rat rises onto its back paws, lifting both front paws and turning its head upward. These parameters were recorded during the trials. At NIEHS, they were also checked for accuracy later by two investigators observing a videotape recording of the open field activity.

In the open field test, separate ANOVAs were calculated for each of the measures (total squares, inner squares, outer squares and vertical). In the passive avoidance test three analyses were performed: a) mean and variance for each of the parameters measured, b) F-ratio test of homogeneity of variances for each of the parameters, and c) Mann-Whitney U-tests on each of the parameters.

Biochemical Measurements

The rats used for biochemical evaluation were sacrificed immediately after exposure by freezing with liquid nitrogen. At NIEHS, the head was removed and stored in a freezer at -70°C. Three days later the brain was extracted. At MRIGCH, the brain was extracted immediately after freezing and stored in liquid nitrogen until the next day. ATPase activity was measured by the technique based on the Fiske-Subarrow method (*12*).

Isolation of the Fractions of Synaptic Membranes from the Cerebral Cortex Synaptosomes. In the U.S., rat cerebral cortex, usually 2 g wet weight, was dispersed in 18 mL of 0.3 M (1:9 w/v) sucrose by using a Potter-Elvehjem type of homogenizer (clearance, 0.25 mm). The pellet obtained after centrifugation at 1912g for 10 min was washed by resuspension in approximately the original volume of 0.3 M sucrose. A fluffy white layer above the pellet was carefully rejected when the supernatant was collected. The combined supernatants were centrifuged at 17,170g for 20 min. The pellet was dispersed in 5 mL 0.3 M sucrose. The suspension was layered over 20 mL 0.8 M sucrose and centrifuged at 17,170g for 20 min (plus 5 min acceleration period). Particles were resolved into the following fractions: (I) a thick white band at the 0.3 to 0.8 M sucrose interface, (II) particles dispersed in

0.8 M sucrose solution, and (III) the pellet. Fraction II was isolated and centrifuged at 47,800*g* for 20 min. The 47,800*g* pellet was suspended in a 4.5 mL of 40 mM Tris, pH 7.4, and used as the synaptosomal enzyme source for Na^+,K^+-ATPase determinations.

In the USSR, cerebral cortex samples were homogenized in 0.32 M sucrose (1:9 w/v) in a glass homogenizer (clearance, 0.25 mm). The homogenate was centrifugated for 10 min at 1000*g*. The supernatant was centrifugated for 30 min at 21,000*g*. The pellet was suspended in 0.32 M of sucrose (pH 7.4–7.6) and homogenized (2–3 fractions). The suspension was layered on the stepped sucrose gradient (5 mL, volume of the cuvette of the spectrophotometer) formed at 14 mL of 1.2 M and 12 mL of 0.8 M sucrose and centrifuged for 60 min at 53,000*g*. The fraction appearing over the 1.2 M sucrose was gathered, diluted with cold distilled water (the water was added slowly at constant mixing) to a sucrose concentration of 0.32 M, and pelleted at 20,000*g* for 30 min. The obtained pellet was suspended in distilled water (2 mL of water per 1 g of original tissue) and left for 30 min at 2 to 4°C. After that, the suspension was centrifuged for 30 min at 20,000*g*. The pellet obtained was suspended again in 0.32 M sucrose and layered upon the stepped gradient, consisting of 12 mL of 1.2 M; 8 mL of 0.8 M sucrose and again centrifuged for 1 hr at 53,000*g*. The fraction appearing over 1 M sucrose (partially gathered from the tube walls) was suspended in 0.32 M sucrose and used for the study. The material obtained was preserved at 4 to 6°C. All the procedures were carried out at 2 to 8°C. The remaining biochemical procedures were carried out identically in the US and USSR except where noted.

Identification of Na^+,K^+-ATPase Activity and the Activity of K^+-Alkaline Phosphatase. Activity of Na^+, K^+-ATPase was identified in the reaction mixture containing 30 mM Tris-HCl (pH 7.4); 4.5 mM $MgCl_2$; 3 mM ATP-Na_2; 100 NaCl; 20 mM KCl. In the control preparations the mixture contained 0.1 mM ouabain as well. The reaction was started by adding the 0.9 mL enzymatic preparation and maintained at 37°C for 20 min and then stopped by addition of the cold trichloracetic acid solution to a final concentration of 5%. Nph (nonorganic phosphor) was identified according to Fiske-Subarrow (*12*).

K^+-alkaline phosphatase activity was identified in 1.5 mL of the following mixture: 30 mL Tris-HCl (pH 7.5); 4.5 mM $MgCl_2$; 3 mM *p*-nitrophenolphosphate; 5 mM KCl. At the beginning of the assay KCl was not present. The reaction was started by adding the enzymatic preparation, the mixture incubated for 10 min at 37°C, and then the reaction was stopped by addition of 3.5 mL 0.2 M NaOH solution. The released *p*-nitrophenol quantity was identified by the spectrophotometer at 390 nm.

Identification of Mg^{2+},Ca^{2+}-ATPase Activity. ATPase general activity, depending on the presence of two-valent Mg^{2+} and Ca^{2+} cations was identified in the media consisting of Tris-HCl (pH 7.4), 6 mM $MgCl_2$, 20×10^{-5} M $CaCl_2$, 3 mM ATP, 0.15 mM ouabain, and about 1.3 to 3.3 mg (US) or 100 μg (USSR) of the enzymatic protein preparation per 1 mL of the incubation mixture [incubation volume, 1.5 mL (US) or 1 mL (USSR); incubation time, 20 min; temperature 37°C]. The reaction was started by adding the protein or ATP, depending on what the aim of the experiment was, and stopped by addition of 0.15 mL 50% trichloracetic acid (US) or 0.25 mL 10% trichloracetic acid (USSR). Mg^{2+}-ATPase was identified in the same way but in the presence of 1 mM of ethyleneglycol-*bis* (β-aminoethylated ether)-*N*,*N*′-trichloracetic acid. Ca^{2+}-ATPase activity was calculated from the general ATPase and the Mg^{2+}-ATPase quantity.

Each of the enzymes measured was analyzed by ANOVA using a randomized complete block design with subsamples where replications represented the blocks and number of animals per group per replication represented the subsamples.

Electrophysiological Measurements

Nine days before exposure, animals used for electrophysiological measurements were anesthetized with sodium pentobarbital or sodium thiopental and implanted with two glass electrodes to record the electrical activity of the cerebral cortex. The electrodes were fashioned from glass pipettes of 2 mm OD and 1 mm ID. The pipettes were heated over a Bunsen burner and stretched to make tips of approximately 20 μm. The electrodes were filled with a mixture of physiological saline and 1 to 2% agar. The electrodes were implanted unilaterally over the occipitotemporal cortex approximately 1 mm apart. The electrodes were cemented in place using epoxy glue on the skull. The electrode impedence was approximately 100 K Ohms.

During the last 3 days of the adaptation period and also during exposure (sham or microwave), polyvinyl chloride tubing filled with 20% sodium chloride and 1 to 2% agar in distilled water was connected to the electrodes. These tubes served as the signal leads during electrophysiological recording. An electrode with a lead attached is shown in Figure 3. Leads were attached to a dowel suspended horizontally from the ceiling and counterbalanced with modeling clay (Fig. 4) to prevent entanglement by the animals' movements.

On the day of exposure, the leads were attached 30 min prior to exposure. EEG recordings were begun 5 min be-

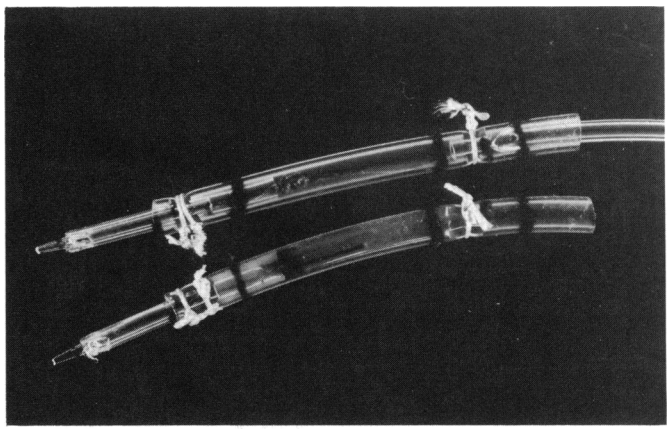

FIGURE 3. EEG electrodes.

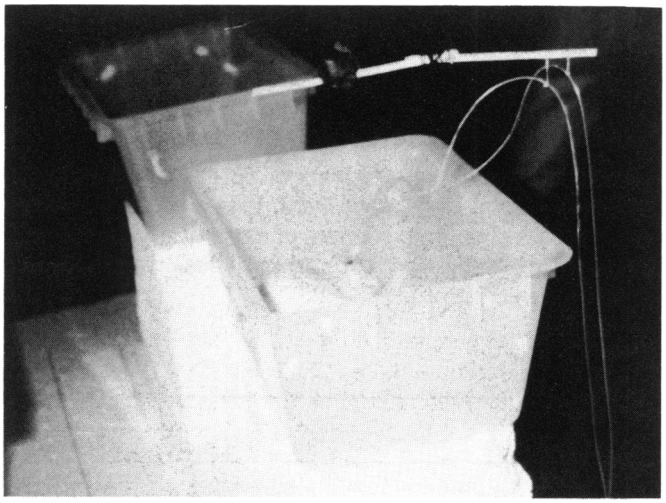

FIGURE 4. Animals with EEG electrodes attached to recording device. This photograph shows the manner in which the leads were prevented from entanglement by attachment to a dowel suspended from the ceiling and counterbalanced with modeling clay.

fore onset of exposure and continued for 5 min after the beginning of exposure. They were then recorded for 2-min periods at hourly intervals. Five minutes before completion of exposure, EEG recordings were again taken for 10 min. The data were recorded on magnetic tape for subsequent power spectral analysis of standard frequencies: delta (1.5–3 Hz), theta (4–7 Hz), alpha (8–13 Hz), $beta_1$ (14–20 Hz), $beta_2$ (21–30 Hz), and gamma (31–70 Hz), as well as total power. Two-minute epochs were analyzed. The units of power were expressed as root mean square volts (V_{rms}). For each frequency band the ratio V_{rms} (for the specified frequency)/V_{rms} (total) was calculated. Overall treatment differences were assessed using a repeated measures ANOVA. If a significant treatment and/or treatment by time interaction was observed, differences across time were assessed using Fisher's LSD test. In addition, ANOVAs were calculated for any overall time difference for each treatment within each frequency band. If such an effect occurred, then significant differences relative to the appropriate premicrowave or presham exposure period were compared using Fisher's LSD test.

Visual evoked potentials (VEP) were recorded 4 min after exposure began and 4 min after its termination. Light flashes of 1.2 msec duration at a frequency of 2 Hz were used. The light was located in the upper corner of the chamber approximately 2.7 m from the center of the animal exposure array. At NIEHS, the source was a Grass model PS22 photostimulator set at 8. This provided an energy output of 0.72 joules per pulse. At MRIGCH it consisted of a 300-watt lamp with an energy output of 0.36 joules per pulse.

The lights in the chamber were turned off for 1 min prior to recording the VEP. At NIEHS, 30 flashes were delivered, and at MRIGCH, 20 flashes were delivered during each recording period. In both cases, however, only 20 responses were averaged. The VEPs were band pass filtered between 1 Hz and 1KHz and averaged for epochs of 320 msec. However, due to the generally poor definition of the VEPs obtained and the inability to exchange raw data, this aspect of the project was not subjected to comparative analysis.

Results

Behavioral Assessments

The data for the open field activity are summarized in Table 2. There were no significant differences between the control and exposed groups. During the first epoch, in particular, the animals in the USSR study tended to be more active than those in the US study, especially with respect to activity in the outer squares. This occurred irrespective of microwave exposure.

In the passive avoidance procedure, only a few of the animals avoided the shock (5/20 in the control and 4/20 exposed in the US study and 2/7 in both groups in the USSR study). The results for those animals that did not avoid the shock are shown in Table 3. The only statistically significant effects were changes in variance. In the US study there were significant increases in variance in the microwave exposed compared to sham exposed data for latency for light avoidance and latency to first entry. With respect to the latter, this was entirely due to one animal. In the USSR study, there was less variability in the microwave-

Table 2. Open field activity.[a]

		Total squares crossed		Inner squares		Outer squares		Rears	
Group	Epoch, min	US	USSR	US	USSR	US	USSR	US	USSR
Control	1	10	16	4	0	6	16	2	4
		(1.8)	(2.6)	(1.0)	(0.1)	(1.2)	(2.7)	(0.3)	(0.9)
	2	8	4	3	0	5	3	2	1
		(1.3)	(0.5)	(0.6)	(0.3)	(1.0)	(0.3)	(0.4)	(0.3)
	3	7	2	3	0	4	2	1	0
		(1.0)	(0.6)	(0.4)	(0)	(0.9)	(0.6)	(0.2)	(0.2)
Exposed	1	8	16	4	2	4	15	2	3
		(1.5)	(3.6)	(0.6)	(0.9)	(1.1)	(3)	(0.5)	(1)
	2	5	5	2	0	3	5	1	1
		(1.1)	(1.1)	(0.5)		(0.6)	(1.1)	(0.3)	(0.2)
	3	5	5	2	0	3	5	1	2
		(1.0)	(0.9)	(0.5)		(0.8)	(1.1)	(0.2)	(1)

[a]Data are averages with SE in parentheses. n = 20 for US and 7 for USSR.

exposed group for time in the dark chamber than the corresponding control group. Again, this effect was entirely due to one animal.

Biochemical Measurements

The biochemical data are summarized in Table 4. In the US study, Na^+,K^+-ATPase activity was significantly less in the microwave-exposed animals than in the sham-exposed animals. There were no other statistically significant results. Note also that the values for all the ATPase activities were much lower in the US study than in the USSR study.

Electrophysiological Measurements

The power spectral analysis of the EEG frequency bands are shown in Figures 5–10. It is evident from the figures that although incidences of statistically significant effects were observed by both groups these were not consistent between groups.

Table 3. Passive avoidance data.[a]

Measure	US		USSR	
	Control	Exposed	Control	Exposed
Latency for light avoidance, sec	6	35	17	11
	(1.7)	(19.8)*	(4.3)	(4.2)
Latency to first entry, sec	7	22	10	17
	(1.7)	(16.1)*	(3.3)	(4.2)
Time in dark chamber per session, sec	220	219	230	290
	(27.2)	(24.9)	(57.5)†	(4.2)
Entries into dark chamber per session	2	2	1	1
	(0.3)	(0.2)	(0.2)	(0)

[a]Data are averages of 15 (US control), 16 (US exposed), and 5 each, USSR control and exposed. Numbers in parentheses represent SE.
*Significantly larger variance than corresponding control.
†Significantly larger variance than corresponding exposed group.

Table 4. Biochemical data.[a]

Enzyme	US		USSR	
	Control	Exposed	Control	Exposed
Na^+,K^+-ATPase	1.273	0.969*	7.47	7.39
	(0.156)	(0.125)	(0.39)	(0.37)
Mg^{2+}-ATPase	0.747	0.766	6.72	6.89
	(0.133)	(0.144)	(0.32)	(0.26)
Mg^{2+},Ca^{2+}-ATPase	0.180	0.178	4.16	4.14
	(0.032)	(0.044)	(0.23)	(0.21)
Alkaline phosphatase	24.82	22.25	17.26	17.22
	(2.36)	(2.10)	(0.82)	(0.67)

[a]Data represent mean and SE (in parentheses) and are expressed in μmole Pi/mg protein/60 min for the ATPases and μmole hydrolyzed/mg protein/60 min for the alkaline phosphatase. n = 20 per group in U.S. study and 18 per group in the USSR study.
*Significantly different from the control group.

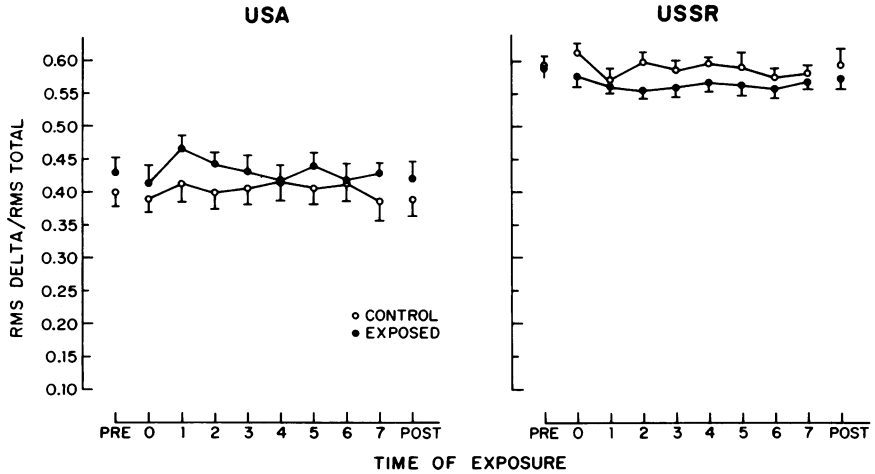

FIGURE 5. Power spectral analysis of the delta band (1.5–3Hz). The ordinate represents the ratio of the root mean square power for the delta band/root mean square of total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3–5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE.

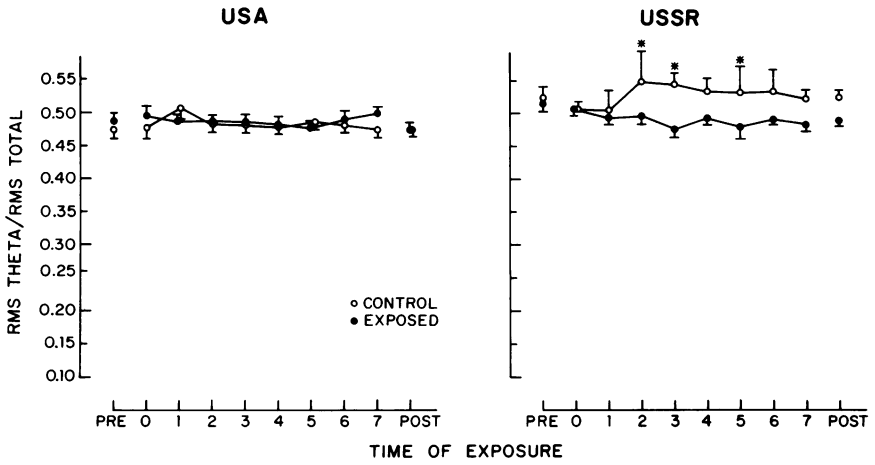

FIGURE 6. Power spectral analysis of the theta band (4-7 Hz). The ordinate represents the ratio of the root mean square power for the theta band/root mean square of total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3-5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE. Asterisk (*) indicates statistically significant difference between the control and exposed groups at $p \leq 0.05$ using Fisher's LSD test.

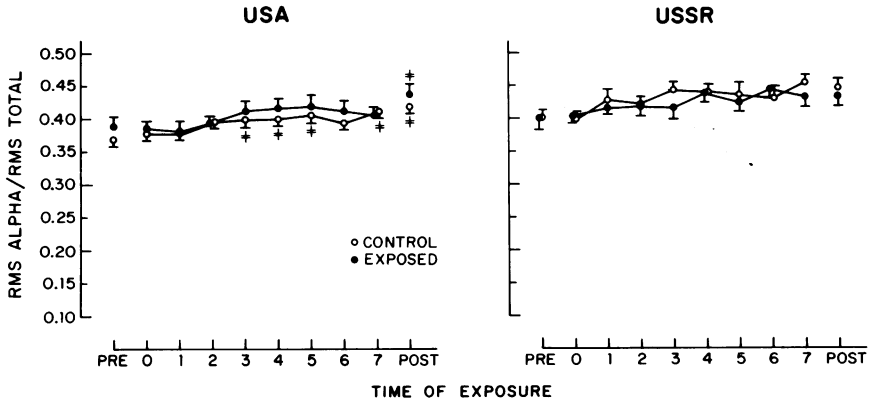

FIGURE 7. Power spectral analysis of the alpha band (8-13 Hz). The ordinate represents the ratio of the root mean square power for the alpha band/root mean square of total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3-5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE. Double cross (≠) below the lines represents a statistically significant difference ($p \leq 0.05$) in the control value at that point compared to the preexposure value for the group. A double cross (≠) above the lines represents a statistically significant difference ($p \neq 0.05$) in the exposed value at that point compared to the preexposure value for that group. Statistical significance was assessed using a Fisher's LSD following an analysis of variance.

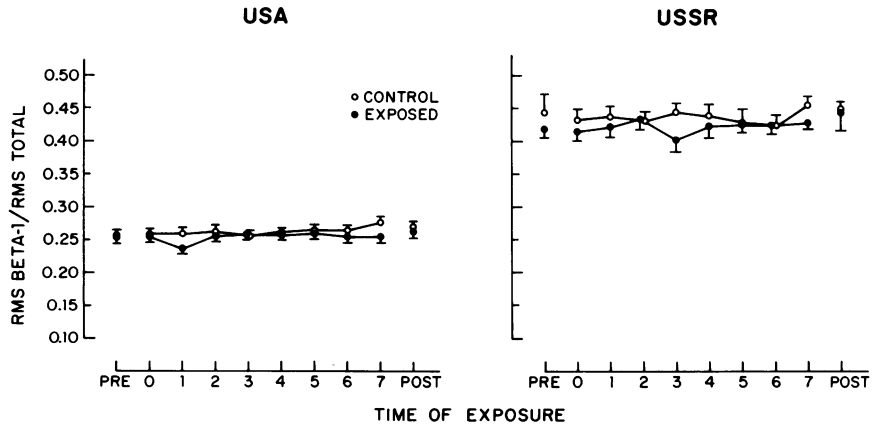

FIGURE 8. Power spectral analysis of the beta 1 band (14-20 Hz). The ordinate represents the ratio of the root mean square power for the beta 1 band/root mean square of total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3-5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE.

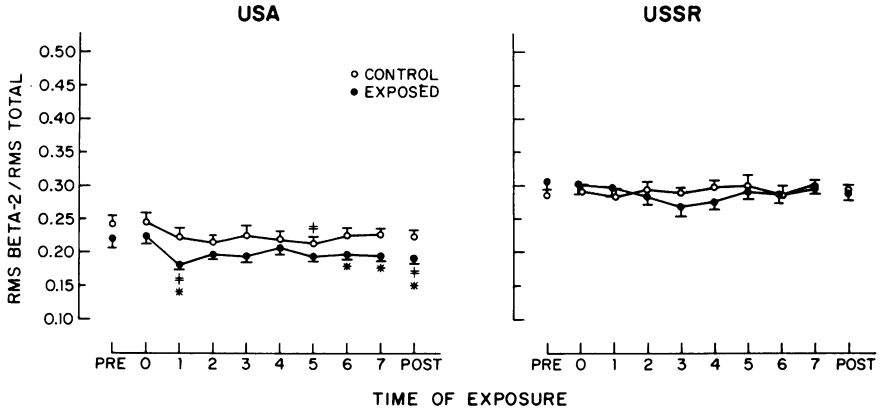

FIGURE 9. Power spectral analysis of the beta 2 band (21-30 Hz). The ordinate represents the ratio of the root mean square power for the beta 2 band/root mean square of total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3-5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE. Asterisk (*) represents a statistically significant difference between the control and exposed groups at $p \leq 0.05$ using Fisher's LSD test. A double cross (≠) below the lines represents a statistically significant difference ($p \leq 0.05$) in the exposed value at that point compared to the preexposed value for the group. A double cross (≠) above the lines represents a statistically significant difference ($p \leq 0.05$) in the control value at the point compared to the preexposure value for that group.

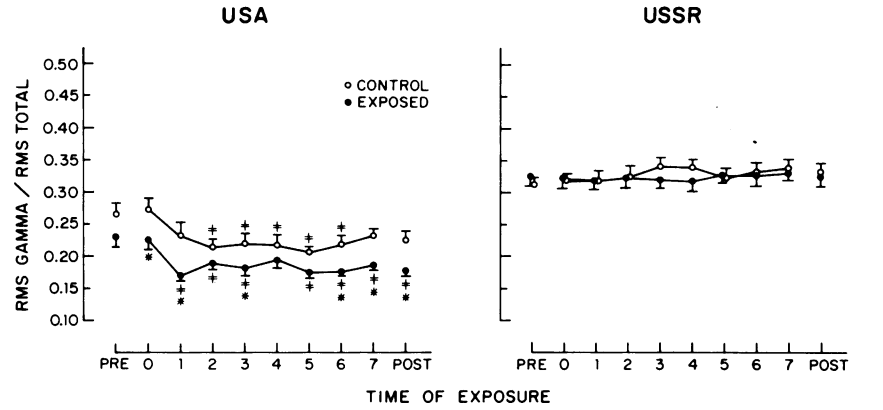

FIGURE 10. Power spectral analysis of the gamma band (31-70 Hz). The ordinate represents the ratio of the root mean square power for the gamma band/root mean square of the total power. The abscissa represents the time of exposure in hours. Pre, recording beginning 5 min before exposure; post, 3-5 min after exposure. Each point represents a 2-min sampling period. The vertical lines represent ± SE. Asterisk (*) represents a statistically significant difference between the control and exposed groups at $p \leq 0.05$ using Fisher's LSD test. A double cross (≠) below the lines represents a statistically signficant difference ($p \leq 0.05$) in the exposed value at that point compared to the preexposure value for that group. A double cross (≠) above the lines represents a statistically significant difference ($p \leq 0.05$) in the control value at that point compared to the preexposure value for that group.

Discussion

The failure of both groups to observe effects in the behavioral tests attributable to microwave exposure was unexpected because previous experience with these tests, using Wistar rats (*5*), had detected differences between microwave-exposed and sham-exposed rats. It is also interesting to note that in both the U.S and USSR studies, all animals entered the dark chamber of the passive avoidance apparatus and remained there for at least 30 sec one or more times during the pretest selection process. Again, this was unexpected, as previous experience with Wistar rats showed that 5 to 10% did not meet this criterion (M. I. Navakatikyan, unpublished results). Also, only a few of the Fischer 344 rats demonstrated a learned response in the passive avoidance apparatus by avoiding the dark chamber on the retest, whereas most of the Wistar rats do demonstrate an avoidance response (M. I. Navakatikyan, unpublished results).

These observations suggest a differential responsiveness of strains and/or inbred versus outbred rats, which needs to be considered when determining the sensitivity of methods for detection of microwave effects on behavior. This is not surprising, but as Ray and Barrett state, "genetic differences among rats are like the weather; some people talk about them but very few researchers do anything about them" (*13*). Suffice it to say that the behavioral literature is replete with examples of behavioral, pharmacological, and biochemical differences among strains of rats (*13-16*); that this should also pertain to microwave effects is to be expected.

With respect to the biochemical measurements, both

groups obtained similar results (namely, no effects of microwave exposure) except for Na^+,Ka^+-ATPase activity. Here the US group obtained a significant depression of Na^+,Ka^+-ATPase activity in the microwave-exposed group, whereas the USSR group found no such effect. This suggests that the effect observed by the U.S. group, while highly statistically significant, may be spurious. On the other hand, there were several differences in the biochemical techniques used between the two groups, particularly in the isolation of synaptosomes, which might account for the observed differences. As is apparent from Table 4, the basal activity of all the ATPases measured was much lower in the US study than in the USSR study. This could be due to a general loss of activity during the isolation procedure and/or subsequent analysis by the US group or due to an isolation of different fractions by the two groups. Two molecular forms of Na^+,K^+-ATPase exist that have different affinities for ouabain (*17*). Attempts have been made to identify the cellular localization of these different enzyme forms, but this has not yet been unambiguously accomplished (*18*). It is known, however, that these two isoenzymes have different sensitivities to certain chemicals (*19,20*) and to hormones such as insulin (*21*) and thyroid hormone (*22*). It is conceivable that microwave irradiation might preferentially affect one of the isoenzymes, either directly or indirectly through alteration of hormonal regulation. Obviously, this can only be resolved by further research.

This study represents our initial attempt at duplicate projects. It is one of the few (and certainly the most extensive) efforts of this type in research on the nervous system effects of microwave radiation. Although much effort was expended in an attempt to ensure that this study was truly a duplicate project, certain differences existed, especially with respect to biochemical analyses. In retrospect, a less ambitious project might have been easier to control. Also, conducting pilot experiments and then meeting to verify adherence to protocols prior to undertaking the complete study would have been useful. Others contemplating such projects might wish to take this into consideration.

Nonetheless, we view this effort as a success in at least two respects. First, for an effort of this magnitude, the incidences of nonduplication of effort were relatively few. Thus, we feel this study demonstrates that duplicate projects of this scale can be conducted. However, the commitment to them must be strong. We remain convinced that such studies are important and necessary. Indeed, the failure of both groups to substantiate the results of the other reinforces this contention. Second, the mutual respect and friendships that resulted from the extensive meetings concerned with developing and refining the protocol and discussing the data were particularly gratifying. We encourage, indeed urge, others to undertake such projects.

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